Global Veterinaria 13 (5): 814-819, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.13.05.86221

Diagnosis of Monieziasis Using Adult *Moniezia expansa* Affinity Partially Purified Antigen

Kadria N. Abdel-Megeed, Soad E. Hassan, Nadia M.T. Abu El -Ezz and Tarek K. Farag

Department of Parasitology and Animal Diseases, Veterinary Research Division,
National Research Center, El Behouse street, Dokki, Giza, Egypt

Abstract: The current study aimed to serodiagnosis of monieziasis among sheep and goats at Beni Suif governorate by using ELISA. Affinity chromatography was adopted to purify *Moniezia* (*M*) *expansa* adult worms extract, which showed more diagnostic activity than eggs extract in the diagnosis of monieziasis by ELISA. Adult, egg extracts and the affinity partially purified antigen were characterized by SDS polyacrylamide gel electrophoresis (SDS- PAGE). *M. expansa* adult worms extract showed 15 bands with molecular weight ranged from 315 -12 KDa, *M. expansa* eggs antigen showed 11 bands with molecular weight ranged from 315 -11.5 KDa, while affinity purified fraction showed simple electrophoretic profile only 3 bands, with molecular weight 164, 88 and 67 KDa. The serodiagnostic value of the *M. expansa* affinity partially purified fraction was checked against antibodies collected from sheep and goats at Beni Suif governorate. Percentage of monieziasis in sheep and goats was found as 69.7%and 74.4% respectively. This study indicates that *M. expansa* affinity partially purified antigen might be successfully used in serodiagnosis for monieziasis in sheep and goats.

Key words: *Moniezia expansa* · ELISA · Purified Antigen · SDS -PAGE · Affinity Chromatography

INTRODUCTION

The genus *Moniezia* is an important parasitic tape worm that has many final hosts such as cattle, sheep, goats and other domestic and wild ruminants. The most important species is *M. expansa* which named the sheep tape worm because of its frequent more in sheep and goats [1-3].

The presence of *Moniezia species* (*spp)* in ruminants can negatively affect their productivity. Lambs are more susceptible to infection with *Moniezia spp* and massive infection with *Moniezia* causes diarrhea and reduced weight gain [4]. Also, it causes gastrointestinal disorders and even death in sheep and goats [5]. So, it constitutes a big problem in sheep raising countries [6-10]. The parasitological method for diagnosis of *Moniezia* infection is performed by fecal analysis in which egg can be detected or often observation of gravid proglottids in feces and anus. Such diagnosis is unreliable because the parasite eggs are not found during prepatent period and do not provide information on percentage of animals which have infection [11].

Serological assays provide the accurate means of premortem diagnosis of *M. expansa* infections in sheep and goats. Specific antigen and sensitive immunodiagnosis tests would be useful aid to control this infection [6]. Information on immunological diagnosis of cestode infection is restricted [12]. Crude antigen preparations can detect anti- *M. expansa* antibodies in sheep [13-16]. But cross reaction with other helminthes were recorded by Njeruh and Gathuma [17], Marks *et al.* [18], Abdel-Rahman *et al.* [19], Abdel-Rahman and Abdel-Megeed [20] and Abdel-Megeed *et al.* [21].

Despite of extensive immunological studies on other tap worm were considered taeniid and *Echinococcus species* reviewed by Kagan [22] Lightowlers [23]; Pan *et al*. [24] and Demeler *et al*. [25], a little work has been reported on isolation and purification of specific antigens from *M. expansa*. Barret *et al.* [26] isolated an intracellular lipid-binding protein from *M. expansa*. The molecular mass of the protein was 7943.6±1.58 Da. An aldehyde reduction enzyme was isolated from mature flukes by chromatofocusing and reactive-red chromatography [27] Also, malate dehydrogenase enzyme of *M. expansa* was

Corresponding Author: Nadia M.T. Abu El-Ezz, Department of Parasitology and Animal Diseases,
National Resarch Center, Postal Code: 12622, Giza, Egypt.

purified by cellulose chromatography [28].Moreover, five fractions were isolated by chromatographic analysis of *M. expansa* crude extract using Sephadex G200 [6].

The infection with monieziasis among animals reached to 28% in some areas of Egypt [29].Moreover, the prevalence of *Moniezia spp.* in sheep was 74% [30]. So, the current research was designed to isolate partially purified antigen from *M. expansa* mature flukes by affinity column chromatography and evaluate the potency of the isolated fraction in serodiagnosis of monieziasis in sheep and goats.

MATERIALS AND METHODS

Samples Collection:

- *Moniezia expansa* worms were collected from small intestine of slaughtered sheep at Beni Suif abattoir.
- *Moniezia expansa* eggs were collected from the mature adult worms.
- 158 Serum samples were collected from Beni Suif governorate (82 sheep samples and 76 goat samples).

Antigens Preparation

***Moniezia expansa* Adult Worm and Egg Extracts:** *Moniezia expansa* adult worms and eggs antigens were prepared separately. Each of them was homogenized in 0.15 M PBS pH 7.2 containing phenyl methyl sulphonyl fluoride (PMSF) and 0.02% NaN_3. Each homogenate was sonicated and then centrifuged at 18000 rpm for 1 h at 4°C. Clear supernatant was collected as crude extract and assayed for protein content according to Lowery *et al.* [31].

Rabbit Hyperimmune Sera: Antibodies against *M. expansa* adult worms antigen were raised in rabbits according to the protocol of Fagbemi *et al.* [32]. Blood collection from rabbits ear vein started 4 at days post last injection. Serum samples were separated and stored at -20 °C untile used.

Affinity Chromatography: Antibodies raised against *M. expansa* adult worms extract were dialyzed against 100 mM $NaHCO_3$ buffer pH 8.3 containing 500 mM NaCl and 0.02% NaN_3 for 3 days according to Abdel-Rahman *et al.* [33]. These antibodies conjugated to Cyanogen Bromide activated Sepharose 4B beeds (CNBr-Sepharose 4B) at the ratio of 2 mg/ml swollen beads by strictly following the manufacture instructions. Then, *M. expansa* adult worms extract was applied to the column coupled with *M. expansa* antisera. Bound fraction was eluted with 50 mM glycine.

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE): *Moniezia expansa* crude adult worms extract, eggs extract and *M. expansa* specific fraction eluted from affinity column were separately electrophresed under reducing conditions SDS-PAGE as described by Lammli [34]. Gels were stained with silver stain according to Wary *et al.* [35]. High and low molecular weight standards were electrophoresed in the same gel.

Enzyme Linked Immunosorbent Assay (ELISA): ELISA was adopted to evaluate immunodiagnostic activity of *M. expansa* crude worms extract and eggs extract activities utilizing two-fold serially diluted natural infected sheep sera. Also ELISA was used to evaluate the potency of the affinity purified fraction in monieziasis serodiagnosis. The assay was performed as described by Santiago *et al.* [36]. The optimum antigens and antibodies concentrations were determined by checkerboard titration. The cut off absorbance values were calculating according to Allan *et al.* [37].

RESULTS

Electrophoretic Profile of *M.expansa* Adult Worm, Egg and Adult Affinity Partially Purified Antigens: Crude adult worm, egg and isolated purified antigens were separately electrophoresed on SDS-PAGE. Adult worms crude antigen were resolved into 15 bands of molecular weights ranged from 315 - 12 KDa (Fig 1, lane3), While, eggs antigen revealed 11bands of molecular weights ranged from 315 - 11.5 KDa (Fig1, lane2). The electrophoretic profile of isolated fraction showed three bands of molecular weights 164, 88, 67 Kda. (Fig 1,lane1).

Evaluation of Immunogenic Activity of *M. Expansa* Adult and Egg Antigens: Immunogenic activity of two antigens (*M.expansa* adults and eggs) was evaluated by ELISA in which natural infected sheep serum was utilized. The immunogenic activity of adult antigen showed potency than eggs antigen as shown in Fig 2. So, adult worms antigen was selected for purification process using affinity column chromatography and its isolated fraction was used for serodiagnosis of moneziasis among sheep and goats.

Table 1: Percentage of monieziasis among examined sheep and goats

Animal	Total No of examined samples	No of +ve samples	No of -ve Samples	Infection %
Sheep	76	53	23	69.7
Goats	82	61	21	74.4

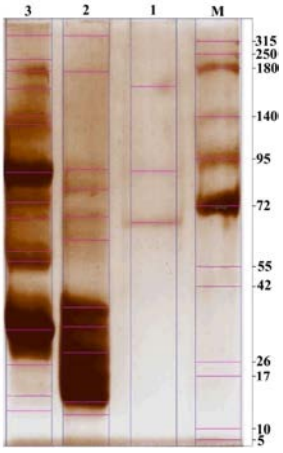

Fig. 1: Electrophoretic profile of *M.expansa* adult, egg and purified antigens. M: Stander molecular weight marker. Lane 1: affinity purified adult antigen. Lane 2: eggs antigen.Lane 3: adult worms antigen

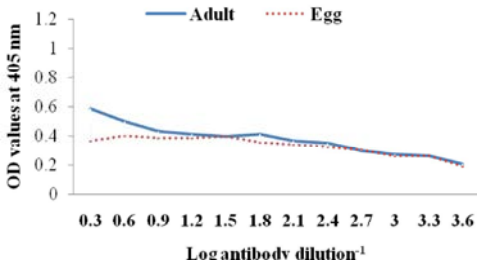

Fig. 2: ELISA evaluation of antigenic activities of *M. expansa* adult worm and egg antigens.

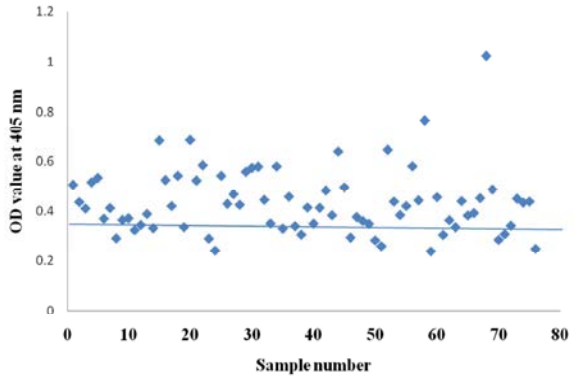

Fig. 3: Scattered graph representing the activity of affinity purified fraction in the diagnosis of moneziasis among sheep.

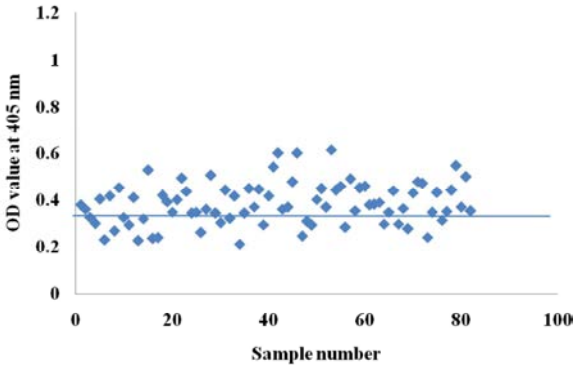

Fig. 4: Scattered graph representing the activity of affinity purified fraction in the diagnosis of moneziasis among goats.

Diagnosis of Moneziasis Using *M. expansa* Affinity Partially Purified Antigen: Serum samples which collected from sheep and goats at Beni- Suif Governorate was investigated by ELISA using *M. expansa* affinity purified antigen and results was depicted in Table 1 and Figs 3 & 4. Infection percentage in sheep was 69.7 while in goats was 74.4.

DISCUSSION

Moniezia expansa could be considered as the most important cestode parasites infesting sheep [14,38]. It is a common ruminant tapeworm of worldwide distribution causing gastrointestinal disorders and even death in sheep and goats [5].

In this study, the immunogenic activity of adult worms antigen showed potency than eggs antigen as depicted by ELISA. Supporting this result, immunization of lambs against monieziasis utilizing adult homogenate of *M. expansa* showed the lowest infection with *M. expansa* [15]. But the multiplicity of parasitic antigen might be considered as the major difficulties in immunological studies of parasites [39]. Immunodiagnosis of monieziasis as well as parasitic infection faces the problem of cross reaction [6] and the success of any diagnostic assay of a given pathogen resides basically on the selection of the appropriate target antigens [40]. Also, many immunological techniques as enzyme linked immunosorbent assay (ELISA) and indirect immunoflorcent have been used in the last few years and provide to be helpful [41 , 42] Depending on these observations together with the data presented in this research, current study adopted a method of affinity column chromatography to purify an immunogenic antigen associated with *M. expansa* adult worms extract

followed by assessment the potency of the isolated fraction in serodiagnosis of monieziasis by ELISA. A total of 53 out of 76 (69.7%) sheep serum samples and 61 out of 82 (74.4%) goat serum samples were positive monieziasis. Although immunodiagnosis is the only reliable assay in monieziasis detection since the prepatent period extended in lambs over a period from 49-55 days [14]. The examination for the parasitic eggs are usually required to increase the accuracy of diagnosis [43].` Among the little attempt of *M. expansa* purification is that of Hassanain and Abdel-Rahman [6] who carried out a method of gel filtration chromatography to purify *M. expansa* adult worms extract. One of five fractions resulted from this method exhibited the most potent activities against rabbits hyperimmune serum by ELISA. This fraction showed success in diagnosis of monieziasis. Also, [27 and 28] were isolated an aldehyde reduction enzyme and malate dehydrogenase enzyme from *M. expansa*, respectively. These studies did not interested with the serodiagnostic value of these enzymes as detected in the current research. Simsek and koroglu [44] detected that seroprevalance of hydatidosis in sheep (at Turkey) was found as 62% by ELISA.Also, the previous authors utilized SDS-PAGE for hydatid fluid. Six bands with molecular weight ranged from 26 - 116 KDa were detected. The variation in results could be attributed to the different localities and species of parasites used in each studies.

In the present study the isolated purified fraction of *M. expansa* was characterized electrophoretically by SDS-PAGE and compared with adult worms and egg extracts of *M. expansa* parasite. The electrophoretic make up of adult and egg extracts showed 15 and 11 bands respectively. While the electrophoretic profile of the isolated affinity purified fraction showed only three bands. Also, in the current study common as well as specific antigens of adult worm and egg extracts were recorded where a common band of almost 88 KDa from adult worms extract and isolated affinity purified fraction was recognized.This 88KDa common band may interpret the potency of adult worms extract than eggs extract as shown by ELISA. Similar result reported by Hassanain and Abdel-Rahman [6] who clarified that five fractions of *M. expansa* isolated by gel filtration chromatography showed simple electrophoretic profile,as judged by SDS- PAGE, compared to the complex profile of crud extract.

In conclusion, the use of affinity purified antigen in serodiagnosis of monieziasis might be of great value in the control and epidemiological studies of monieziasis in sheep and goats. Further investigation needed to evaluate its potency as vaccine candidate.

REFERENCES

1. Schuster, R., 1998. Epidemiology of sheep monieziasis. Praktische Tierarzt, 79: 357 - 363.
2. Al-Qureishy, S.A., 2008. Prevalence of cestode parasites in sheep slaughtered in Riyadh city, Saudi Arabia. J. Egypt. Soc. Parasitol. 38: 273-280.
3. Jie, L.M., P. Yong-Shual, P. Zhu, F. Jian, G. Wang, M.A. Hui-hai, W. Zhu and C. Ning, 2013. Survey on the prevalence of intestinal parasites in sheep. China Animal Husbandry and Veterinary Medicine, 4: 201-205.
4. Elliott, D.C., 1986. Tapeworm (*Moniezia expansa*) and its effect on sheep production: the evidence reviewed. N. Z. Vet. J., 34: 61-65.
5. Yan, H., X. Bo, Y. Liu,Z. Lou, X. Ni, W. Shi, F. Zhan, H. Ooi and W. Jia, 2013. Differential diagnosis of *Moniezia benedeni* and *M. expansa* (Anoplocephalidae) by PCR using markers in small ribosomal DNA (18S rDNA). Acta Vet. Hung., 61: 463-472.
6. Hassanain, M.A. and E.H. Abdel-Rahman, 2000. Evaluation of partially purified *Moniezia expansa* worm antigen in diagnosis of monieziasis. Beni-Suef. Vet. Med. J., 10: 147-156.
7. Mazyad, S.A. and H.I. El-Nemr, 2002. The endoparasites of sheep and goats and shepherd in North Sinai Governorate, Egypt. J. Egypt. Soc. Parasitol., 32: 119-126.
8. Agyei, A.D., 2003. Epidemiological studies on gastrointestinal parasitic infections of lambs in the coastal Savanna regions of Ghana. Trop. Anim. Health Prod., 35: 207-217.
9. Haridy, F.M., H.A. Dawoud and T.A. Morsy, 2004. Efficacy of Commiphora molmol (Mirazid) against sheep naturally infected with *Moniezia expansa* in Al-Santa Center, Gharbia Governorate, Egypt. J. Egypt. Soc. Parasitol., 34: 775-782.
10. Chilton, N.B., M.G. O'Callaghan, I. Beveridge and R.H. Andrews, 2007. Genetic markers to distinguish *Moniezia expansa* from *M. benedini* (Cestoda: Anoplocephalidae) and evidence of the existence cryptic species in Australia. Parasitol. Res., 100: 1187 - 1192.
11. Roepstorff, A., 1998. Natural *Ascaris suum* infections in swine diagnosed by coprological and serological (ELIZA) method. Parasitol. Res., 84: 237-243.
12. Lightowlers, M.W., 1990. Cestode infections in animals: immunological diagnosis and vaccination. Rev. Sci. Tech. Off. Int. Epiz., 9:463 - 487.

13. Haralampidis, S.T., 1987. ELISA in the seroepidemiology of parasitoses of sheep and goats. Bulletin of the Hellenic Vet. Med. Society, 38: 215 -223.
14. Połec, W., 1990. Immunological studies on lambs experimentally infected with *Moniezia expansa*. Acta Parasitologica Polonica, 35: 333-339.
15. Polec, W., 1991. The effect of immunization of lambs naturally infected with Moniezia sp. Acta Parasitologica Polonica. 36: 207-210.
16. Polec, W., 1992. Studies on the immunological response of lambs immunized and spontaneously infected by *Moniezia spp*. Acta Parasitologica Polonica. 37: 93 - 97.
17. Njeruh, F.M. and J.M. Gathuma, 1987. Serodiagnosis of livestock hydatidosis by the use of indirect hemagglutination test (IHA) and the enzyme-linked immunosorbent assay (ELISA). Bull. Anim. Hlth. Prod. Africa, 35: 124-129.
18. Marks, N.J., D.W Halton, A.G. Maule, G.P. Brennan, C. Shaw, V.R. Southgate and C.F. Johnston, 1995. Comparative analyses of the neuropeptide F (NPF)- and FMRFamide-related peptide (FaRP)-immunoreactivities in *Fasciola hepatica* and *Schistosoma spp*. Parasitology, 110 : 371-381.
19. Abdel-Rahman, E.H., K.N.Abdel-Megeed and M.A. Hassanian, 2000. Structural characterization and immunologicalization of egg antigens cross-react with *Toxocara vitulorum*, *Fasciola gigantica* and *Moniezia expansa* mature flukes. J. Egypt. Soc. Parasitol. 30: 581-591.
20. Abel-Rahman, E.H. and K.N. Abdel-Megeed, 2005. Cross-protection induced by cross-reactive antigen against *Fasiola gigantica* and *Trichinella spiralis* infections. J. Egypt. Soc. Parasitol., 35: 281-294.
21. Abdel-Megeed, K.N., E.H. Abdel Rahman and A.M. Derbala, 2005. Identification and diagnostic evaluation of cross-reactive antigen between hydatid cyst fluid of *Echinococcus granulosus* and *Trichinella spiralis* larval extract. J. Egypt. Soc. Parasitol., 35: 497-509.
22. Kagan, I.G., 1968. A review of serological tests for the diagnosis of hydatid disease. Bull. Org. Mond. Santé. Bull. Wld. Hlth. Org., 39: 25-37.
23. Lightowlers, M.W., 1996. Vaccination against cestode parasites. Int. J. Parasitol., 26: 819 - 882.
24. Pan, D., A.K. Bera, S. Bandyopadhyay, S. Das, T. Rana, S.K. Das, S. Bandyopadhyay, B. Manna and D. Bhattacharya, 2011. Molecular characterization of antigen B2 subunit in two genotypes of *Echinococcus granulosus* from Indian bubaline isolates, its stage specific expression and serological evaluation. Mol. Biol. Rep., 38: 2067-2073.
25. Demeler, J., E. Schein and G. Von Samson-Himmelstjerna, 2012. Advances in laboratory diagnosis of parasitic infections of sheep. Vet. Parasitol., 189: 52-64.
26. Barret, J., N. Saghir, A. Timanova, K. Clarke and P.M. Brophy, 1997. Characterization and properties of an intracellular lipid-binding protein from the tapeworm *Moniezia expansa*. Eur. J. Biochem. 250: 269 - 275.
27. Brophy, P.M., P. Crowley and J. Barrett, 1990. A novel NADPH/NADH-dependent aldehyde reduction enzyme isolated from the tapeworm *Moniezia expansa*. FEBS Lett., 263: 305 - 307.
28. Sanchez-Moreno, M., P.Teiada, M.A. Garcia-Ruiz and M. Monteoliva, 1988.Cu-Zn superoxide difmutase activity in *Moniezia expansa*. Wiadomosci-Parazytologiczne, 34: 113-124.
29. Hassan, M.G. and M.M. El-Bahi, 1992. Comparative study on enteric parasites infesting farm and field cattle and buffaloes at Suez governorate. Assiut Veterinary Medical Journal, 27: 88-98.
30. Bashtar, A.R.1., M. Hassanein, F. Abdel-Ghaffar, K. Al-Rasheid, S. Hassan, H. Mehlhorn, M. Al-Mahdi, K. Morsy and A. Al-Ghamdi, 2011. Studies on monieziasis of sheep I. Prevalence and antihelminthic effects of some plant extracts, a light and electron microscopic study. Parasitol Res., 108(1): 177-186.
31. Lowry, O.H., N.J. A.L. Rosenbrough, R.J. Farr and Randall, 1951. Protein measurement with the Folin Phenol Reagent. J. Biol. Chem., 193: 265-275.
32. Fagbemi, B.O., I.O. Obarisagbon and J.V. Mbuh, 1995. Detection of circulating antigen in serum of *Fasciola gigantica* infected cattle with antibodies reactive with a Fasciola-specific 88-Kda antigen. Vet. Parasitol., 58: 235-246.
33. Abel-Rahman, E.H., Nadia M.T. Abu El-Ezz and K.N. Abdel-Megeed, 2005. *Trichinella spiralis* Affinity purified antigen Based diagnosis and immunoprophylaxis J. Egypt. Soc. Parasitol., 35: 379-393.

34. Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680 - 685.
35. Wray, W., T. Boulikas, V.P. Wray and R. Hancock, 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. 118: 197-203.
36. Santiago, N.G., Hillyer, M. Garcia-Rosa and M.H. Morales, 1986. Identification of functional *Fasciola hepatica* antigens in experimental infections in rabbits. Am. J. Trop. Med. Hyg., 35: 135-140.
37. Allan, J.C., P.S. Craig, J. Garcia Noval, F. Liu, D.Y. Wang, H. Wen, P. Zhou, R. Stringer, M. Rogan and E. Zeyhle, 1992. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology. 104: 347 - 356.
38. Hassanain, M.A., A.M. Bashter, F.A. Abdel Ghafar and S.E. Hassan, 2014. Light and ultrastructure modifying the endocrine character of *Moniesia expansa* (Rud. 1805, Cestoda Cyclophylidea) interproglottidal gland. The Journal of Zoology, 1: 7-11.
39. Derbala, A.A., 1999. The use of identified specific antigen for serodiagnosis of *Toxocara vitulorum* infection in buffalo calves.Vet. Med. J. Giza, 47: 89:96.
40. Abdel-Megeed, K.N. and E.H. Abdel Rahman, 2003. Comparative immunodiagnostic approach of Toxocaiasis in buffalo calves. J. Egypt. Soc. Parasitol., 33: 473-484
41. Nunez, G.G., S.N. Costantion and S.M. Vemturiello, 2003. Immuno-parasitological parameters of the intestinal phase of Trichinellosis in rats. Parasitol., 126: 321-325.
42. Arab, R.M.H., Nadia M.T. Abu El-Ezz, Nabila S. Deghidy,Walid S.A. Awed and Noha M.F. Hasssan, 2013. Protective Value of *Haemonchus contortus* Adult Worm Purified Antigen Against Haemonchosis in Sheep. Global Veterinaria. 11: 614-621.
43. Tak, I.R., J.S. Dar, B.A. Ganai, M.Z. Chishti, R.A. Shahardar, T.A. Tantry, Y. Nizam and S.A. 2014. Comparative analysis of different immunological techniques for diagnosing fasiolosis in sheep: Areview. Biotechology and Molecular Biology Reviews, 9: 21-25.
44. Simsek, S. and E. Koroglu, 2004. Evaluation of enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunoelectrotransfer blot (EITB) for immunodiagnosis of hydatid diseases in sheep. Acta Tropica. 92 : 17-24.